AUGUST 1996

AUSTRALIAN QUARANTINE REQUIREMENTS FOR THE IMPORTATION OF BOVINE SEMEN FROM THE UNITED STATES OF AMERICA

1. GENERAL

1.1 Each consignment of semen must be accompanied by a valid "Permit to Import Semen/Embryos into Australia".

The permit must be obtained, prior to the export of the semen, from the Australian Quarantine and Inspection Service (AQIS) office in the State of import. A processing fee will be charged for the permit.

1.2 Each consignment must also be accompanied by an Animal Health Certificate signed by an Official Veterinarian and the Semen Collection (or AI) Center Veterinarian. An Official Veterinarian is a civil service veterinarian or a specially appointed veterinarian as authorized by the United States Department of Agriculture (USDA). The Semen Collection Center Veterinarian must be accredited by the USDA to supervise the collection of semen.

The Animal Health Certificate must conform to the Office International des Epizooties (OIE) International Animal Health Code (Code) Model Certificate No. 3. The Certificate is to be stamped on each page with an Official stamp.

****An Animal Health Certificate to be used is included in this document.**

1.3 The semen must meet the requirements specified in Section 2 of this document and this must be certified in the Animal Health Certificate.

Reference is made through Section 2 to Office International des Epizooties (OIE) International Animal Health Code (the OIE Code) Articles defining requirements for disease freedom, and to the OIE Code Appendix 4.2.1.2. relating to the Hygienic Collection and Handling of Bovine Semen.

The relevant OIE Code Articles are at Attachment 1.

OIE Code Appendix 4.2.1.2 is at Attachment 2.

1.4 The consignment must be shipped to the Australian importer care of AQIS.

1.5 Fees may be applied by AQIS to cover costs associated with the selection, collection, testing, processing, or quarantine of the semen and any Australian Government veterinary supervision of the consignment.

1.6 Conditions of importation may be varied or reviewed at any time at the discretion of the Australian Director of Animal and Plant Quarantine (the Director).

2. CERTIFICATION

The required certification is included in the Animal Health Certificate in this document, under item IV. SANITARY INFORMATION.

3. SPECIAL CONDITIONS

AQIS may, with the concurrence of the Chief Veterinary Officer in the State of import, allow the importation of semen collected from bulls that have not been tested for Johne's disease. Applications should be made to AQIS through the AQIS office in the State of import.

4. IMPORTERS/AGENTS RESPONSIBILITIES

4.1 It is the responsibility of the importer to arrange for any other health certification or health testing of donors to meet State entry requirements of Breed Society requirements (eg testing or certification for inherited disease or defects) or that he/she may require for any other purpose.

4.2 The importer or agent must nominate a person who will be accessible to AQIS officers and who will accept that responsibility for ensuring that all import requirements are met.

5. POST ARRIVAL

5.1 The consignment will be held by AQIS until a Quarantine Officer has checked the certification and conducted an audit of the contents of the shipping container.

5.2 If a consignment arrives in Australia without the correct certification, with the seals on the transport containers broken or in any other way not having met these requirements, the consignment may be retained in quarantine, returned to the country of origin or destroyed without recompense.

Health Certificate No. _____
(Valid only if USDA Veterinary Seal
appears over certificate number)

ANIMAL HEALTH CERTIFICATE

Import Permit Number: .....
Species and Category: BOVINE SEMEN
Exporting Country: UNITED STATES OF AMERICA
Ministry/Department of: AGRICULTURE
Service: ANIMAL AND PLANT HEALTH INSPECTION SERVICE.

Region/District/Province/State: .....

I. INFORMATION CONCERNING THE DONORS AND THE SEMEN.

Name and Breed	Herd Book No.	Date of Collection	Straw Identification	Number of Straws

Total number of straws: .....

II. ORIGIN OF THE SEMEN.

Exporter - Name: _____
Address : _____
_____
_____
Place of origin of the semen, if different from above: _____
_____

III. DESTINATION OF THE SEMEN

Consignee - Name:_____
Address : _____
_____
_____

Nature and identification of the means of transport: _____

Health Certificate No. _____
(Valid only if USDA Veterinary Seal
appears over certificate number)

IV. SANITARY INFORMATION

I, ..., the USDA Accredited Veterinarian responsible for the supervision of the semen collection center (.....) hereby certify that:

1. The USA is recognized by the OIE as a foot and mouth disease (FMD) *free country where vaccination is not practised* (Article 2.1.1.2) and meets the OIE Code definitions of country freedom for rinderpest (Article 2.1.4.2), contagious bovine pleuropneumonia (Article 2.1.6.2), lumpy skin disease (Article 2.1.7.2) and Rift Valley fever (Article 2.1.8.2).

2. The donors were, at the time of semen collection, part of the resident herd at a semen collection center (AI center) which complies with 'CSS (Certified Semen Services) Minimum Requirements for Disease Control of Semen Produced for AI'.

   They were examined and tested prior to entry, during isolation before entering the resident herd, and before semen release, for tuberculosis, brucellosis, leptospirosis, bovine viral diarrhea virus, bovine genital campylobacteriosis and trichomoniasis in accordance with CSS requirements and found free from these diseases.

3. Bovine brucellosis and bovine tuberculosis

   (a) Bovine brucellosis and bovine tuberculosis are compulsorily notifiable in the USA.

   *(b) The AI center has been *officially free* from bovine brucellosis and bovine tuberculosis as defined by OIE Code Articles 3.2.1.1 & 3.2.3.1 respectively,

or

   *Certified Brucellosis-Free and Accredited (tuberculosis free) by the USDA, APHIS for five years prior to the final collection of semen for this consignment,

or

   *is located in a Class Free (brucellosis) and Accredited Free (tuberculosis) State or area.

   **Note: Delete above options not utilized*

4. Vesicular stomatitis has not been reported within 80 kilometers of the AI Center during the period from two months prior to first collection of semen for this consignment until one months after the final collection of semen for this consignment.

5. Enzootic bovine leucosis: Each donor **either:**

originates from an EBL *free herd* (as defined by the OIE Code Article 3.2.4.2) or a herd recognized by USDA, APHIS as free from EBL, and the resident herd at the AI Center is recognized as free from EBL.

or

was tested for EBL, by an approved serological test of a serum sample collected at least 21 days but no more than 90 days after the final collection of semen for this consignment, with negative results. Date of test: _____

6. An aliquot of not less than 0.5 ml of processed semen from each donor, pooled, if there is more than one collection, from at least two collections for this consignment, was tested at a USDA approved laboratory by cell culture inoculation for infectious bovine rhinotracheitis (IBR) virus with negative results after 3 passages. Date of test: _____

7. Each donor was tested for Johne's disease (paratuberculosis) by an absorbed enzyme-linked immunosorbent assay (ELISA), with negative results, after the first collection of semen for this consignment but not more than 180 days after the final collection for this consignment. Date of test: _____

8. Bluetongue

either:

*The semen was collected between 15 January and 15 May from donors resident for at least 60 days in an AI Center located in a USDA designated bluetongue low incidence State.(AK, CT, DE, HI, IN, ME, MD, MA, MI, MN, NH, NJ, NY, ND, OH, PA, RI, VT, WI, WV)

or

*At least 14 days before the first collection and again between 21 days and 90 days after the final collection of semen for this consignment, serum samples were collected from each donor and tested at a USDA approved laboratory for bluetongue antibodies using competitive ELISAs or agar gel immunodiffusion tests, or virus neutralization tests for each serotype of bluetongue known to occur in the USA, with negative results in each case.

or

*At least 14 days before the first collection of semen for this consignment, and thereafter at intervals not exceeding 7 days (the last being on the last day of semen collection for this consignment from that donor), blood samples were collected **from each donor** and examined, at a laboratory approved by the USDA for this procedure, with negative results for bluetongue virus (BTV) by:

either

- inoculation of at least 0.25 ml packed red blood cells into a BTV sero-negative sheep which was tested and found to be BTV sero-negative 28 days later;

or

- culture in embryonating chicken eggs inoculated intravenously with washed, lysed and diluted red blood cells followed by examination of the embryos for virus by passage in cell cultures by an approved method (such as inoculation into BHK21 cell culture);

or

direct antigen or nucleic acid detection (polymerase chain reaction (PCR) testing) using a technique of proven high sensitivity.

**Note: PCR testing is not an option without prior written AQIS approval*

9. Epizootic haemorrhagic disease of deer (EHD)

either

The semen was collected between 15 January and 15 May from donors resident for at least 60 days in an AI Center located in a USDA designated bluetongue low incidence State.

or

At least 14 days before the first collection and again between 21 and 90 days after the final collection of semen for this consignment, serum samples were collected from each donor and tested at a USDA approved laboratory for EHD antibodies using agar gel immunodiffusion tests, virus neutralization tests for other tests approved by AQIS, for each serotype of EHD known to occur in the USA, with negative results in each case.

or

At least 14 days before the first collection of semen for this consignment, and thereafter at intervals not exceeding 7 days (the last being on the last day of semen collection for this consignment from that donor), blood samples were collected from each donor and examined, at a laboratory approved by the USDA for this procedure, with negative results for EHD virus by:

either

-virus isolation by inoculation of cell culture;

or

-direct antigen or nucleic acid detection (PRC testing) using a technique of proven high sensitivity.

(Note: PCR testing is not an option without prior written AQIS approval)

10. Semen collection, processing and storage

- The semen was collected and processed in accordance with the CSS Requirements and the

recommendations of the OIE Code Appendix 4.2.1.2 parts C and D.

- Each semen receptacle was indelibly marked with the identification details of the donor and the date of collection or a code from which this information could be determined.

- The semen was stored in fresh liquid nitrogen in sterilized containers in which no biological material, other than semen, embryos or ova eligible for importation into Australia, was held.

- The period between the first and last collection of semen for this consignment from each donor was not more than 90 days.

- The semen transport container was sealed, with an official seal, prior to shipment. Marking on seal: .....

_____	_____
Accredited Veterinarian (date)	Endorsing Federal Veterinarian (date) (Valid only if USDA Veterinary Seal appears over signature)
_____	_____
_____	_____
_____	_____
Name and address	Name and address

Attachment 1
OIE International Animal Health Code definitions as referenced:

Article 3.2.1.1, bovine brucellosis (Brucella abortus)
Herd officially free from bovine brucellosis

To qualify as officially free from bovine brucellosis, a herd of cattle shall satisfy the following requirements:

1) be under official veterinary control;

2) contain no animal which has been vaccinated against bovine brucellosis during at least the past three years;

3) only contain animals which have not showed evidence of bovine brucellosis infection during the past six months, all suspect cases (such as animals which have prematurely calved) having been subjected to the necessary laboratory investigations;

4) all cattle over the age of one year (except castrated males) were subjected to serological tests with negative results performed twice at an interval of 12 months. This requirement is maintained even if the entire herd is normally tested every year or testing is conducted in accordance with other requirements established by the Veterinary Administration of the country concerned;

5) additions to the herd shall only come from herds officially free from bovine brucellosis. This condition may be waived for animals which have not been vaccinated, come from a herd free from bovine brucellosis, provided negative results were shown following a buffered *Brucella* antigen test and the complement fixation test during the 30 days prior to entry into the herd. Any recently calved or calving animals should be retested after 14 days, as tests are not considered valid in female animals which have calved during the past 14 days.

Article 3.2.3.1, Bovine tuberculosis

Country or *part of the territory of a country officially free* from bovine tuberculosis

To qualify as officially free from bovine tuberculosis, a country or part of the territory of a country shall satisfy the following requirements:

1) bovine tuberculosis is compulsorily notifiable in the country;

2) 99.8% of the herds in the considered geographical area have been officially free from bovine tuberculosis for at least the past three years as disclosed by periodic testing of all cattle in the area to determine the absence of bovine tuberculosis (periodic testing of all cattle is not required in an area where a surveillance program as described in paragraph 4 below, reveals that

at least 99.9% of the cattle have been in officially tuberculosis-free herds for at least six years);

3) cattle introduced into a country or part of the territory of a country officially free from bovine tuberculosis must be accompanied by a certificate from an *Official Veterinarian* attesting their compliance with Article 3.2.3.9. or the criteria set out in this Article;

4) a country or part of the territory of a country officially free from bovine tuberculosis must have a *Veterinary Administration* which should be able to trace and test the herd of origin of any reactor to a tuberculin test disclosed after removal from the considered territory. Also animals which at a post-mortem examination carried out by a veterinarian in an abattoir or elsewhere disclosed gross pathological lesions of tuberculosis which where necessary can be confirmed by established methods of microscopical-biological or cultural examination. In addition, such a country or part of the territory of a country officially free from bovine tuberculosis must have in place a surveillance program to ensure the discovery of bovine tuberculosis should the disease be present in the country or part of the territory of a country, through slaughter monitoring and/or tuberculin testing.

Herd officially free from bovine tuberculosis

To qualify as officially free from bovine tuberculosis, a herd of cattle shall satisfy the following requirements:

a) the herd is in a country or part of the territory of a country officially free from bovine tuberculosis; or

b) all cattle in the herd:
1) show no clinical signs of bovine tuberculosis;

2) over six weeks of age, have shown a negative result to at least two official tuberculin tests carried out at an interval of six months, the first test being at six months following the eradication of bovine tuberculosis from the herd;
3) showed a negative result to an annual tuberculin test to ensure the continuing absence of bovine tuberculosis;

c) cattle introduced into the herd:
1) have been certified by an Official Veterinarian as having shown a negative result to the tuberculin test during the 30 days prior to entry into the herd; and/or
2) were kept in a herd officially free from bovine tuberculosis.

Article 3.2.4.2, EBL *free herd*

1) Qualification

To qualify as free from EBL, a herd must satisfy the following requirements:

a) there has been no evidence of EBL either clinical, post mortem, or as a result of a diagnostic test for EBL within the previous two years;

b) all animals over 24 months of age have been subjected to a diagnostic test for EBL on two occasions with negative results, at an interval of not less than 4 months during the preceding 12 months;

c) animals introduced into the herd after the first test have originated from a free herd, or fulfilled the conditions of Article 3.2.4.3.

2) Maintenance of free status

For a herd to maintain its EBL free status, the animals in the herd over 24 months of age on the day of sampling must be subjected to a diagnostic test for EBL with negative results at intervals of no more than 36 months and the conditions in paragraphs 1)a) and 1)c) above continue to be fulfilled.

3) Suspension and restoration of free status

If in an EBL free herd any animals react positively to a diagnostic test for EBL or a virological test (under study) for bovine leukosis virus, the status of the herd shall be suspended until the following measures have been taken:

a) the animals, which have reacted positively, and their progeny since the last negative test, must be removed from the herd immediately. However, any animal within the progeny which are subjected to a PCR test with negative results (under study) may be retained in the herd;

b) the remaining animals must be subjected to a diagnostic test for EBL carried out as described in paragraph 1)b) above with negative results at least three months after removal of the positive animals and their progeny.

Attachment 2
OIE International Animal Health Code, Appendix 4.2.1.2., Chapters B & C

B. MANAGEMENT OF BULLS

The objective is the daily care of bulls to ensure a satisfactory state of cleanliness, particularly of the lower and ventral parts of the chest.

1. The bull should be kept under hygienic conditions at pasture, or if this is not possible in tethered or loose housing. If kept tethered, the litter must be kept clean and renewed as often as necessary.

2. The coat of the bull should be kept clean and generally short.

3. The length of the tuft of hairs at the preputial orifice, which is invariably soiled, should be cut to about 2 cm. The hair should not be removed altogether, because of its protective role. If cut too short, it may set up an irritation of the preputial mucosa.

4. The animal should be brushed regularly, and where necessary on the day before semen collection, paying special attention to the underside of the abdomen.

5. In the even of obvious soiling, there should be careful cleansing, with soap or a detergent, of the preputial orifice and the adjoining areas, followed by thorough rinsing and drying off.

6. In the case of an abnormally large preputial orifice or abnormalities within the cavity which might be accompanied by an invasion of micro-organisms, the preputial sac may be washed out before semen collection. Sterile saline solution is introduced several times into the prepuce using a catheter attached to a siphon tube. This precaution is vital if the subsequent ejaculate is to be tested for any pathogenic bacteria which might be present.

7. When the bull is brought of its stall into the collection room, the technician must make sure that the bull is clean, and that it is not carrying any litter or particles of feed on its body or its hooves, for such materials are always heavily contaminated.

C. SEMEN COLLECTION

1. The floor of the mounting area should be easy to clean, to dry and to disinfect. A dusty floor should be avoided.

2. The hindquarters of the teaser, whether a dummy or a live teaser animal, must be kept clean. A dummy must be cleaned completely after each period of collection. A teaser animal must have its hindquarters cleaned carefully before each collecting session. It is advisable to repeat the cleansing upon each change of bull, particularly in the case of soiling by defecation.

Plastic covers are poorly accepted by bulls, and are not generally used in practice.

3. The hand of the person collecting the semen must not come into contact with the bull's penis. The wearing of disposable, and preferably sterilized gloves is advisable to provide extra protection should the bull move unexpectedly.

4. it is necessary to clean the artificial vagina completely before each collection. It should have been dismantled beforehand, its various parts washed, rinsed and dried, and kept protected from dust. The inside of the body of the device and the cone should be sterilized before reassembly using approved sterilization techniques to such as those involving the use of 70% ethyl or 98-99% isopropyl alcohol, ethylene oxide or steam. Once assembled it should be kept in a cupboard which is regularly cleaned and disinfected.

5. The lubricant used should be sterile and packed in tubes. The rod used to spread the lubricant must be sterile and should not be exposed to dust between successive collections.

6. It is recommended that the artificial vagina not be shaken after ejaculation, as otherwise lubricant and debris may pass down the cone to join the contents of the collecting tube.

7. When successive ejaculates are being collected, a new artificial vagina should be used for each mounting. The vagina should also be changed when the bull has inserted its penis without ejaculating.

8. The collecting tubes must be sterile, and the recommended method of sterilization is heating in an oven at 180ºC for at least 30 min. They should be sealed while awaiting use, for example by a plug of sterile cotton wool, and kept in a sterile box or cupboard until required.

9. After collection, the tube should be left attached to the cone and within its sleeve until it has been removed from the collection room for transfer to the laboratory.

October 1993

CSS (Certificed Semen Services) MINIMUM REQUIREMENTS FOR DISEASE CONTROL OF SEMEN PRODUCED FOR AI

The "CSS Minimum Requirements for Disease Control of Semen Produced for AI" provides a minimum standard for the health monitoring and disease surveillance of bulls prior to entry, during an isolation period and throughout residency at an AI center. This is a comprehensive standard for those disease proven to be a significant threat to be seminally transmitted by artificial insemination. Furthermore, it outlines proper sanitary procedures and includes requirements for the addition of appropriate antibiotics to semen and extender to control specific microorganisms. The goal of these requirements is to protect the health of the herd in which the semen is used.

GENERAL SANITARY CONDITIONS

1. Semen collection equipment which comes in contact with the bull or his secretions or excretions shall be thoroughly disinfected after each use or, a separate set of equipment shall be assigned to each animal.

2. Scrupulous cleanliness or new disposable plastic gloves shall be used by the collecter on each bull to assure that his hands cannot serve as a means of transmitting infectious, contagious material from bull to bull.

3. The laboratory used for semen processing shall be fully enclosed and partitioned from bull housing and semen collection areas, and structured to provide for hygienic handling and storage of semen.

4. The health tests to be conducted in accordance with the following requirements shall be conducted in a manner generally consistent with the procedures described in "The Recommended Uniform Diagnostic Procedures for Qualifying Bulls for the Production of Semen" as published by the American Association of Veterinary Laboratory Diagnosticians (AAVLD) or other diagnostic procedures recognized as being at least equal to the AAVLD published procedures.

5. Attention shall be given to liquid nitrogen refrigerators returning from foreign countries not declared free of foot and mouth disease by USDA, to determine if they have been disinfected at the port of entry. If they have been properly disinfected, there will be a tag attached indicating this fact. If disinfection has not been done, the USDA/APHIS veterinarian in the state involved shall be notified and appropriate action shall be taken immediately to have the refrigerators properly disinfected.

MOUNT ANIMALS

Mount animals (teasers) used at semen collection shall be submitted to the same regimen of periodic tests as bulls in semen production. Areas of contact by the erect penis or of genital secretions upon the hair coat or skin of a mount shall be effectively and thoroughly disinfected

between successively mounting bulls.

PRE-ENTRY

The following pre-entry tests and examinations shall be conducted for each bull within thirty (30) days prior to permitted entry into the isolation facilities of the AI business:

1. Physical Examination: A physical examination shall be conducted by an accredited veterinarian to determine that the bull does not display any clinical symptoms of any infectious, contagious disease.

2. Tuberculosis: Intradermal tuberculin test, the result of which shall be negative.

3. Brucellosis: The official blood serum test for brucellosis as used in the state in which the bull is located or which complies with applicable regulations if the bull must be shipped interstate.

4. Bovine leptospirosis: A blood test for serotypes L. pomona, L. hardjo, L. canicola, L. icterohaemorrhagiae, and L. grippotyphosa. Any animal exhibiting a significant titer may be subjected to a second blood test within two to four weeks after the first. An end or limiting titer may be run on both samples. Cattle exhibiting a stabilized low titer on both tests may be considered satisfactory to enter the isolation facility.

ISOLATION

Each bull shall be held in isolation throughout the period of time necessary to conduct the below listed tests before the bull is permitted to enter the facilities occupied by resident bulls and before any semen from the bull is released for use.

For purposes of these requirements isolation shall mean that the bull is housed in facilities which are effectively separated from facilities occupied by resident bulls and that all equipment used to handle the bulls for semen collection, feeding and watering, and cleaning the facilities occupied by the bulls shall not be used for both isolation and resident herds. Further, semen collection areas for bulls in isolation shall be effectively separated from areas used for resident bulls.

1. The following tests shall be conducted for all bulls:

    a. Tuberculosis: One intradermal tuberculin test, the result of which shall be negative. This test shall be conducted at least sixty (60) days after the date of the pre-entry test for tuberculosis.

    b. Brucellosis: One blood serum tube or plate agglutination test (1:50) and one complement fixation (CF) test with negative results. These serological tests shall be conducted not sooner than thirty (30) days after the date of the pre-entry test for brucellosis.

Should the bull show an agglutination response to the brucellosis test at the 1:50 dilution or higher, or, should the bull have a positive response at 1:10 or higher or an anti-complementary test result to the brucellosis CF test, it is recommended that another serological test (tube or plate agglutination and/or CF test) be conducted. Furthermore, other USDA official brucella tests (card, rivanol, BAPA, PCFIA) may be performed. A negative result on retest or on one or more of these additional official brucella tests may permit the bull a negative brucella classification, but final classification remains the prerogative of the state veterinary officials.

c. Bovine leptospirosis: Tests for serotypes L. pomona, L. hardjo, L. canicola, L. icterohaemorrhagiae, and L. grippotyphosa. These blood serum tests shall be conducted not sooner than thirty (30) days after the date of the pre-entry tests for leptospirosis.

d. Bovine viral diarrhea virus (BVDV): All bulls and teaser animals entering CSS approved AI centers after January 1, 1991 must be tested for persistent BVDV infection with negative results before entry into the AI Center's Resident Herd. No animal from which BVDV has been isolated is to have semen distributed or to be used as a mount (teaser) until demonstrated free of persistent infection as described in paragraph d.(iv).

The following test methods and schedules are to be used to test for persistent BVDV infection. (Any deviations in testing practices must have the prior approval of the CSS Service Director.)

i. Diagnostic test: The animal must be subjected to one virus isolation test performed in bovine cell culture with a negative result as demonstrated by staining of the cell culture by immunofluorescence (FA) or immunoperoxidase (IP) methods.

ii. Diagnostic specimen: For animals less than 6 months of age the BVDV isolation test must be conducted on the mononuclear cells of a whole blood specimen. for animals 6 months of age or older, the BVDV virus isolation test may be performed on whole blood, serum, or semen.

iii. Confirmation of persistent BVDV infection: If BVDV is demonstrated by FA or IP in cell culture, the animal is to be isolated from other cattle and retested in not less than 21 days by inoculation of bovine cell cultures with an appropriate specimen (see item d.ii). Demonstration of BVDV a second time is considered diagnostic of persistent infection and the animal is not eligible to enter the resident herd of the CSS-approved AI center.

iv. Confirmation that an animal is not persistently infected: Animals from which BVDV has been isolated or demonstrated must remain in isolation apart

from other cattle until proven free of BVDV by 2 consecutive negative virus isolation tests conducted at least 10 days apart and performed on the appropriate specimen (item d.ii).

Bulls from which BVDV has been isolated but are later proven to be free of persistent infection as stated above must have semen that was collected and processed within the 30 days preceding and following the date of virus isolation, subjected to BVDV isolation tests with negative results from each collection code before distribution.

2. The following tests shall be conducted for all bulls before their semen is released for use. However, if the bulls are not of semen producing age at the beginning of the isolation period these tests may be conducted after the isolation period is completed:

   a. Brucellosis: One semen plasma agglutination test (1:25) with a negative result.

   b. Bovine Campylobacteriosis (Vibriosis): Preputial material shall be cultured and examined for Campylobacter fetus venerealis, the results of which shall be negative. As an alternative procedure, the preputial material may be examined using the fluorescent antibody (FA) technique as a screening test. Any positive FA test shall be followed by a culture of preputial material, the results of which shall be negative.

      Bulls may be placed on the following variable testing schedule:

Age of Sire when entering isolation	Minimum number of tests (at weekly intervals)
Less than 12 months	3
12 months and over	6

   c. Bovine venereal trichomoniasis: A series of culture microscopic examinations of preputial material collected from the fornix shall be negative. The frequency of testing shall be the same as that listed under ISOLATION 2.b. Bovine campylobacteriosis (vibriosis).

3. All semen shall be treated with the antibiotics gentamicin, tylosin, and Linco-spectin (GTLS) as described by Shin, et al (1), Lorton, et al (2) and Lorton, et al (3). Details of the procedures to be used are listed in Appendix 1.

RESIDENT HERD

Once a bull has completed the isolation period as outlined above, he may enter the resident herd where he shall continue to be tested in accordance with the below listed test procedures so long as his semen is collected for use in artificial insemination.

1. The following tests shall be conducted for all bulls:
   a. Tuberculosis: Negative to the official intradermal tuberculin test at intervals of six (6) months.

   b. Bovine brucellosis: Serological tests [tube or plate agglutination (1:50) and CF] shall be conducted at intervals of six (6) months. (Refer to ISOLATION 1.b. Brucellosis for additional information.)

   c. Bovine leptospirosis: Tests for serotypes L. pomona, L. hardjo, L. canicola, L. icterohaemorrhagiae, and L. grippotyphosa shall be conducted at intervals of six (6) months.

   d. Bovine venereal trichomoniasis: A single culture test for bovine venereal trichomoniasis shall be conducted at intervals of six (6) months.

   e. Bovine campylobacteriosis (vibriosis): Single culture tests of preputial material for bovine campylobacteriosis (vibriosis) shall be conducted and found negative at intervals of six (6) months. As an alternative procedure, the preputial material may be examined using the fluorescent antibody (FA) technique as a screening test. Any positive FA test shall be followed by culture of preputial material, the result of which shall be negative.

   Antibiotics shall be added to all processed semen as described above (refer to ISOLATION 3.)

2. All bulls in the resident herd shall be maintained in continuous isolation from all cloven hoofed animals that have not completed all of the test procedures outlined herein with negative results to all such tests throughout the period in which semen is collected from the bull for use in artificial insemination. At any time that an individual bull from the resident tested herd is permitted contact with an untested animal he shall be removed immediately from the resident tested herd and shall not be permitted re-entry until such time as he has completed another cycle of isolation and the tests prescribed therefor, except as provided for in paragraph 3 below.

3. It is not required that a bull temporarily held out of semen production be tested for bovine trichomoniasis and bovine campylobacteriosis (vibriosis) provided he is at a location effectively separated from the resident herd. However, he shall be maintained in a herd which otherwise meets all conditions of a resident herd. The routine testing regimen as defined for the resident herd must be resumed immediately prior to the release of semen processed after his return to production.

ANTIBIOTICS AND SEMEN PROCESSING

1. Antibiotics will be added to the neat semen and extender to provide effective microbiological control of:

Mycoplasmas
Ureaplasmas
Haemophilus somnus
Campylobacter fetus subsp venerealis

2. Effective microbiological control is the condition in which the number of organisms potentially present under pathological conditions are reduced to subclinical levels (i.e., below the threshold of infectivity).

3. An acceptable protocol is the treatment of semen and extender with the antibiotics gentamicin, tylosin, lincomycin and spectinomycin (GTLS) as described by Shin, et al (1), Lorton, et al (2) and Lorton, et al (3). Details of the procedures to be followed are described in Appendix 1.

4. Acceptable alternative protocols must provide effective microbiological control (of organisms in 1 above) based on scientific evidence, submitted to Certified Semen Services, Inc.

REFERENCES

(1) Shin, S.J., D.H. Lein, V.H. Patten and H.L. Ruhnke. 1988. A New Antibiotic Combination for Frozen Bovine Semen. 1. Control of Mycoplasmas, Ureaplasmas, Campylobacter fetus subsp. venerealis and Haemophilus somnus. Theriogenology. 29:577.

(2) Lorton, S.P., J.J. Sullivan, B.Bean, M. Kaproth, H. Kellgren and C. Marshall. 1988. A New Antibiotic combination for Frozen Bovine Semen. 2. Evaluation of Seminal Quality. Theriogenology. 29:593.

(3) Lorton, S.P., J.J. Sullivan, B. Bean, M. Kaproth, H. Kellgren and C. Marshall. 1988. A New Antibiotic Combination for Frozen Bovine Semen. 3. Evaluation of Fertility. Theriogenology. 29.609.

APPENDIX 1, GTLS ANTIBIOTIC PROCEDURES/CONDITIONS

A. Antibiotics/Stock Solutions

(1) Antibiotics:

a. Gentamicin sulfate: powder, micronized, non-sterile, U.S.P. (veterinary grade)
100 grams per bottle

b. Tylosin: labeled as Tylan Soluble, product of Elanco Products Company
100 grams per bottle

c. Linco-Spectin: product of the Upjohn Company
20 ml per vial, each ml contains 50 mg lincomycin and 100 mg spectinomycin

NOTE: Antibiotics obtained from some sources have not been tested and may contain deleterious agents that may harm or kill sperm cells. For recommended sources, contact Certified Semen Services.

(2) Stock solutions of individual antibiotics (gentamicin and tylosin) may be prepared and stored separately at 5°C for eight days or stored frozen in LN vapor for up to six months. Linco-Spectin as supplied by distributor should be maintained at 5°C after it is opened.

(3) Stock solutions of individual antibiotics will be combined on day of use, and not held over.

(4) Extenders must be used on the day the combined antibiotics are added.

B. Neat Semen Treatment

(1) 100 μg of tylosin, 500 μg gentamicin and 300/600 μg of Linco-Spectin dissolved in .02 ml of double distilled sterile water will be added and carefully mixed with each ml of neat semen.

NOTE: All of the antibiotic concentrations expressed herein are for active units of antibiotic. Potency values may vary between batches of antibiotic. Therefore, amounts of raw material have to be adjusted for each batch in order to obtain the required concentrations of active antibiotic.

(2) The addition of these antibiotics should be scheduled so as to allow a three to five minute time period for the antibiotics to be in contact with the neat semen before the addition of any extender.

C. Non-Glycerol Fraction of Extender

(1) All non-glycerol fractions of any of the five extenders listed below will be prepared to contain the following concentrations of antibiotics before being added to semen:

tylosin	100 μg per ml
gentamicin	500 μg per ml
Linco-spectin	300/600 μg per ml